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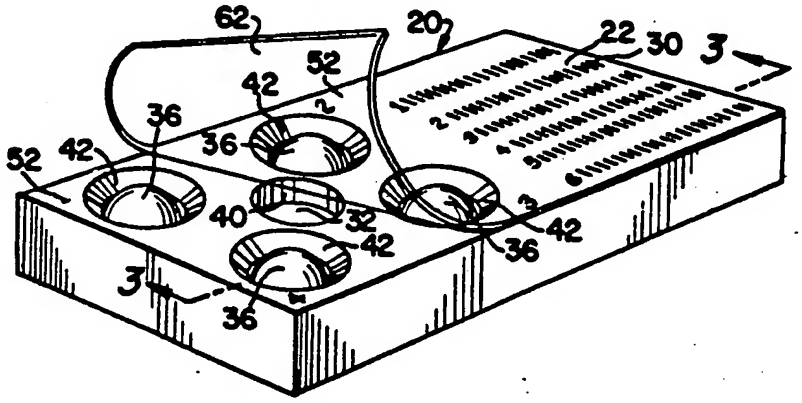

(57) Abstract

A test apparatus for facilitating the performance of chemical, clinical diagnostic, immunoassay and like test. The apparatus includes a housing (22) and a sample receiving area or well (32) defined in the housing and adapted for receiving a sample or specimen. A plurality of rupturable containers (36) are mounted in recesses (34) formed in the housing. Each rupturable container contains the necessary quantity f the specific reagent (38) required for the test. A duct or channel (44) fluidly connects each recess (34) with the sample receiving area or well (32). A sharp spike, or member with a sharp edge (46) is provided in the housing in operative association with each rupturable container (36) to permit an operator to rupture the containers (36) in the sequence required for the test, and thereby to cause the liquid reagents (38) to flow to the sample receiving area or well (32).

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DIAGNOSTIC TEST KIT

BACKGROUND OF THE INVENTION

1. Cross-Reference to Related Application

The present application is a continuation-in-part of application Serial Number 728,255, filed on April 29, 1985 in the United States Patent Office.

2. Technical Field

The present invention is in the field of diagnostic kits and devices. More particularly, the present invention is directed to an apparatus or device which contains the necessary reagents for the performance of diagnostic, immunoassay, and like tests on a sample or specimen, and which facilitates the performance of such tests.

3. Technical Background

Numerous diagnostic, immunoassay, and like tests are commonly used in the clinical diagnostic and related fields. In most tests, a sample or specimen is treated sequentially with predetermined quantities of different reagents. Often, the sample must be incubated with a given reagent for predetermined periods of time before the next reagent is added. Many immunoassay tests utilize an antibody bound to a solid medium. Usually, in such immunoassay tests several "washing" or "rinsing" steps are required to remove excess reagents which are not bound to the antibody.

Positive results of diagnostic immunoassay and like tests are usually indicated by visually perceptible changes, such as color changes, which occur in the treated sample or specimen in the final steps of the tests. As is well known, commonly used clinical diagnostic, immunoassay, and like tests usually test the blood, urine, or other body fluids or tissues, for the presence of certain chemicals, hormones, antigens, pathogens, or abnormal cells.

In some clinical diagnostic, immunoassay, or like tests, a mere "positive" or "negative" evaluation of the sample or specimen is desired with reference to the presence or absence of the chemical, hormone, antigen, or pathogen which is evaluated by the test. In many other clinical diagnostic tests, a quantitative evaluation of the sample or specimen is made with regard to the level of chemical, hormone, antigen, or pathogen. The quantitative evaluation is often made by spectrophotometric measurement of the color intensity, and/or of other physical property or properties of the sample or specimen.

As is well known by those skilled in the art, many clinical diagnostic laboratories employ numerous skilled technicians to perform, on a daily basis, a very large number of diagnostic, immunoassay, and like tests of the above-described nature. Although the tests are often "routine", they require precision and accuracy. Under the present economic conditions existing in the United States of America, and in most parts of the industrialized world, the skilled laboratory technicians' labor usually contributes a very significant portion to the total cost of "routine" medical diagnostic tests.

In light of the foregoing, many efforts were made in the prior art to provide test kits and devices which strive to simplify "routine" clinical diagnostic, immunoassay, and like tests, facilitate their performance in terms of ease of manipulation of laboratory equipment, and reduce the possibility of human error in the performance of the tests. The apparatus or device of the present invention represents a significant advance over the prior art with regard to the above.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a diagnostic test kit apparatus which contains, in a readily usable form, the proper amounts of all, or nearly all, reagents which are necessary for the performance of the test.

It is another object of the present invention to provide a diagnostic test kit apparatus which renders performance of multi-step diagnostic tests relatively simple, and reduces the possibility for human error in the performance of the tests.

It is still another object of the present invention to provide a diagnostic test kit apparatus for the performance of immunoassay tests using an antibody bound on a solid phase, which meets the above-noted objectives and contains the solid phase bound antibody.

It is yet another object of the present invention to provide a simplified, relatively human error-free process for the performance of multi-step clinical diagnostic, immunoassay, and like tests.

The foregoing and other objects and advantages are attained by a test kit apparatus which includes a housing and a sample receiving area, or well, defined in the housing and adapted for receiving a sample or specimen. At least one, but preferably a plurality of rupturable containers, are mounted in the housing. Each container contains the predetermined amount of one of the specific reagents which are required for the performance of the particular diagnostic or immunoassay test for which the kit is designed.

A duct or channel connects each area below each container with the sample receiving area or well, where the sample or specimen is located. A pointed protrusion, or other like member, preferably integrally molded with the housing, is provided in operative association with each container, to enable a technician operator to rupture the container when addition of the specific reagent is required in the test. The liquid or powder reagent content of each container flows through the respective duct or channel into the sample receiving area or well where the test reactions occur.

In embodiments of the device used for immunoassays utilizing a solid bound antibody, a filter paper or similar member supporting the antibody is disposed in the sample

receiving area or well. A porous plate is disposed below the antibody supporting member to permit the excess reagents and wash solutions to drain into a liquid absorbing material or into a liquid reservoir space disposed below.

In alternative embodiments, the containers, instead of being ruptured or sheared by an object, may contain a weakened wall section whereby they can be ruptured by application of pressure only.

The features of the present invention can be best understood, together with further objects and advantages, by reference to the following description, taken in connection with the accompanying drawings, wherein like numerals indicate like parts.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of a first preferred embodiment of the test kit apparatus of the present invention;

Figure 2 is a partial top view of the first preferred embodiment shown on Figure 1, the view showing a transparent protective cover member covering the top of the embodiment;

Figure 3 is a cross-sectional view taken on lines 3,3 of Figure 1;

Figure 4 is a partial top view of the first preferred embodiment shown on Figure 1, without the transparent protective cover member;

Figure 5 is a partial view of the first preferred embodiment, the view being taken on lines 5,5 of Figure 3;

Figure 6 is a cross-sectional view taken on lines 6,6 of Figure 4;

Figure 7 is a partial cross-sectional view of a second preferred embodiment, the view showing an intact container for a liquid reagent incorporated in the embodiment;

Figure 8 is a partial cross-sectional view of the second preferred embodiment, the view showing the container ruptured by a shearing device incorporated in the embodiment;

Figure 9 is a partial cross-sectional view of a third preferred embodiment, the view showing an intact container for a liquid reagent incorporated in the embodiment;

Figure 10 is a perspective view of a plastic or like sheet to which plastic bubble containers for liquid reagents are affixed in accordance with the present invention;

Figure 11 is a partial perspective view of a fourth preferred embodiment;

Figure 12 is a partial top view of the fourth preferred embodiment;

Figure 13 is a perspective view of a fifth preferred embodiment;

Figure 14 is a partial top view of a sixth preferred embodiment;

Figure 15 is a cross-sectional view of a seventh preferred embodiment;

Figure 16 is a partial top view of an eighth preferred embodiment;

Figure 17 is a partial top view of a ninth preferred embodiment, and

Figure 18 is a partial top view of a tenth preferred embodiment.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The following specification, taken in connection with the drawings, sets forth the preferred embodiments of the present invention. The embodiments of the invention disclosed herein are the best modes contemplated by the inventor for carrying out his invention in a commercial environment, although it should be understood that various modifications can be accomplished within the scope of the present invention.

Referring now to Figures 1-6 of the appended drawings, the first preferred embodiment 20 of the diagnostic test apparatus of the present invention is disclosed. It should be noted at the outset that the present invention is generally and broadly directed to test kits or apparatus

which can be adapted to a large variety of chemical, clinical diagnostic, immunoassay, and like tests. The invention is not limited by the type of the test performed in the apparatus. The specific examples of diagnostic or immunoassay tests which are made in the present description should, therefore, be considered exemplary, rather than limiting in nature.

The test apparatus or kit of the present invention includes a housing 22 wherein, in accordance with the present invention, all, or nearly all, reagents required for performing the test are contained in the specific amounts required in the test. The housing 22 is preferably made of molded plastic and comprises a bottom plate 24, an upper plate 26, and an intermediary member 28, best shown on the cross-sectional views of Figures 3 and 6. The foregoing component parts of the housing 22 are preferably attached to one another by sonic welding, gluing, or the like processes which readily adapt themselves to mass manufacturing.

A portion of the top surface of the upper plate 26 of the housing 22 preferably carries indicia 30 providing step-by-step directions for performing the specific test. It should be understood that the indicia 30, that is, the directions for performing the specific test, depend on the nature of the test. The indicia or directions 30 are only schematically shown on Figure 1.

The housing 22 defines a first sample receiving area 32 and a plurality of additional areas 34 for receiving a plurality of small containers 36 which contain the liquid reagents 38 required for the test. More specifically stated, and with reference to Figures 1-6 of the appended drawings, the sample receiving area 32 is a well, formed in the intermediate member 28 of the housing 22, and the additional areas 34 each comprise an additional, although preferably smaller, well or depression also formed in the intermediate member 28 in the housing 22.

In some embodiments (not shown) of the present invention, there may be only one additional area for

receiving only a single container for a liquid or powdery reagent. Such an embodiment is, of course, preferably used in a test where only a single reagent is required.

Returning now to the description of the first preferred embodiment 20, the sample receiving area or well 32 is located below an opening 40 in the upper plate 26. Moreover, each of the additional wells or depressions 34 in the intermediate member 28 are located below respective openings 42 in the upper plate 26.

Each of the wells or depressions 34 are connected to the main, sample receiving well 32 by a duct or channel 44 so that liquid or powdery reagents can flow from the wells or depressions 34 to the main sample receiving well 32. As is apparent from the drawings, the ducts or channels 44 are integrally molded with the intermediate member 28 of the housing 22, and comprise only a small volume relative to the liquid capacity of either the small containers 36 or of the main sample receiving well 32.

A sharp protrusion or spike 46 is located substantially in the center of each well or depression 34. In the herein-described specific embodiments, the spikes 46 are integrally molded with the intermediate member 28 of the housing 22. Each container 36 comprises a sealed plastic bubble. Each bubble 36 contains the predetermined amount of one of the specific liquid reagents 38 required in the specific diagnostic, immunoassay, or like test for which the test kit is designed. Each plastic bubble 36 is located substantially below the respective opening 42 in the upper plate 26 of the housing 22, and above the respective spike 46 of the corresponding well 34.

Preferably, as in the herein-described preferred embodiment 20, the plastic bubbles 36 are affixed to a substantially flat plastic sheet 48, which is shown in the cross-sectional views of the drawing Figures, and also on Figure 10. For convenience and ease of manufacture, the plastic bubbles 36 are preferably formed in a film of plastic 49, and the plastic sheet 48 serves as their closure. The

plastic sheet 48 has a cut off corner 50 which serves, together with a matching shoulder (not shown) in the housing 22, as a key during assembly of the test kit. This assures that the plastic sheet 48, bearing the plastic bubbles 36, can be properly mounted in the housing 22 in only one orientation.

Indicia 52, such as the arabic numerals 1, 2, 3, and 4, are located adjacent to each of the openings 42 below which the plastic bubble containers 36 are disposed. These indicia or numerals 52 identify the specific liquid reagent 38 contained therein. The numerals 52, together with the directions 30 (and possibly in combination with other instructions regarding the specific test to be performed), provide the information on the basis of which even a semi-skilled technician (not shown) is able to perform the specific chemical, diagnostic, or immunoassay test for which the particular test apparatus is specifically designed.

Thus, in order to perform the test, an operator technician (not shown) places the sample or specimen 54 in the sample receiving well 32. As it will be readily understood by those skilled in the art, the sample 54 may be a liquid, such as a body fluid, blood, blood serum, or urine. In other than medical diagnostic tests, the sample 54 may be some other liquid, such as water, as, for example, in tests designed for drinking water, swimming pool water, or the like. The sample or specimen 54 may also be solid or semi-solid material, such as a tissue sample which is required in many diagnostic or pathology laboratory tests. The sample 54 may also be a soil sample for soil analysis.

In the event the test apparatus is designed for an immunoassay test using a solid phase bound antibody (not shown) of the type where a component of the sample or specimen binds to the antibody, where reagents are sequentially added to the solid phase for additional binding, and where excess reagents are "rinsed" away from the solid phase, then the apparatus of the present invention also includes the solid phase bound antibody (not shown).

More specifically, and with reference to Figure 6 showing the first specific embodiment 20 in cross-section, a porous plate 56 is located below the main sample receiving well 32, and a liquid absorbing material 58 is located below the porous plate 56 in the housing 22 just above the bottom plate 24. The porous plate 56 is of the type which permits slow drainage of liquid, and may be made of materials commonly used for this purpose in the laboratory equipment and related arts. The liquid absorbing material 58 preferably comprises cellulose, the exact nature of the absorbant material 58, as well as of the porous plate 56, not being critical for the purposes of the present invention. The total amount of the absorbant material or absorbant padding 58 is, however, such that it is capable of absorbing all, or virtually all, liquid reagent 38 which gradually drains through the porous plate 56.

Referring still particularly to the cross-sectional view of Figure 6, a filter paper or filter membrane 60 is shown disposed above the porous plate 56. The filter paper or membrane 60 comprises the bottom of the main sample receiving area or well 32. For immunoassay tests of the above-specified type, the filter paper or membrane 60 contains the bound antibody which is required in the specific test.

The performance of the specific steps of an immunoassay or like test using the apparatus of the present invention should be readily apparent in light of the foregoing description. After the sample or specimen 54 is placed on the filter paper or membrane 60 in the main well 32, the technician operator (not shown) waits the requisite amount of time (if such time is necessary) to permit binding of the sought-after component (not shown) to occur to the solid supported antibody (not shown). Preferably, the sequence of steps to be followed, including waiting periods between the successive steps, are described in the directions contained in the indicia 30.

As the directions 30 call for successive addition of specific reagents 38, or rinse solutions, the technician operator (not shown) presses, through the respective opening 42 in the housing 22, the specifically identified plastic bubble container 36 against the spike 46. This action ruptures the plastic sheet 48 that forms the closure of the plastic bubble container 36 and releases its liquid reagent 38. The liquid reagent or rinse solution 38 flows through the narrow duct or channel 44 into the main sample receiving area or well 32, where the requisite chemical or immunological reaction may occur within the pores of the filter paper or membrane 60. Excess reagent or rinse solution 38 gradually drains through the porous plate 56 into the absorbant padding 58 below. In this regard it is noted that virtually the entire reagent 38 content of the plastic bubble or container 36 is forced to flow to the main sample receiving well 32 when the operator (not shown) manually presses the plastic bubble 36. The plastic sheet 48, which forms the closure of the plastic bubble container 36, comprises the upper closure for the ducts 44. This is best shown on the cross-sectional view of Figure 6.

The progress of the test, as well as the final color or other visually perceptible change indicating the outcome of the test, is observed through the opening 40 located above the main sample receiving well 32.

Figures 1 and 2 show a protective plastic cover 62 disposed on the top surface of the upper plate 26 of the housing 22. The protective plastic cover 62 is an optional component of the apparatus of the invention. It protects the plastic bubble containers 36 from accidental rupture, either by a sharp external object, or through inadvertently pressing against the spikes 46, during shipping and handling. The protective cover 62 may carry the indicia 30. The protective cover 62 is preferably transparent, and is preferably affixed to the housing 22 with a pressure sensitive adhesive (not shown) which permits its removal by peeling. Figure 2 shows a separate portion 64 of the protective sheet 62, which can

be replaced on the housing 22 after the sample or specimen 54 has been added. This permits agitation of the sample with the reagents or rinse solutions in the main sample receiving well 32, by mild shaking, rocking, or the like. In certain tests this may be desirable. It is noted that, unless a distinction is necessary and is made in the present description, the terms "reagents" and "rinse or wash solutions" are used interchangeably.

Referring now to Figures 7 and 8, a second preferred embodiment 66 of the test apparatus of the present invention is disclosed. The second preferred embodiment 66 differs principally from the first preferred embodiment 20 in the manner the plastic bubble containers 36 are ruptured to release their respective liquid reagent 38 contents. As is shown in Figures 7 and 8, in the second preferred embodiment 66 each plastic bubble container 36 incorporates a relatively narrow section 68. The narrow section 68 is disposed below a vertically movable member or plug 70 placed in a substantially vertically formed bore 71 in the housing 22. When it is desired to rupture the plastic bubble container 36, the plug 70 is pressed downwardly to shear off the narrow section 68. A circumferential groove or notch 72 in the plug 70 permits the liquid reagent to flow around the plug 70 through the respective duct 44, to the main sample receiving well 32.

Figure 9 shows a third preferred embodiment 74 of the test apparatus of the present invention. In this embodiment, the plastic bubble containers 36 include a weakened wall section 76 designed to rupture under internal pressure which is attained when the bubble container 36 is finger-pressed by a technician-operator (not shown). After the weakened wall section 76 is ruptured, the liquid reagent 38 is released into the main sample receiving well 32, substantially as in the above-described embodiments.

Figures 11 and 12 show a fourth preferred embodiment 78 of the test apparatus of the present invention. This embodiment is primarily designed for use in tests where it is

desirable to remove the sample or specimen after the test has been completed, for example, for the purposes of conducting spectrophotometric or radiation counting assays. As is shown on Figures 11 and 12, in the fourth preferred embodiment 78, the main sample receiving area or well 32 is located in a tray 80, which is inserted through an appropriate opening 82 into the housing 22. As a further option, the sample receiving well 32 may be divided into sections 84. In certain tests, the specimen may be placed in one or two of the three sections, and a known control or controls may be placed in the remaining section or sections 84. In such cases, the test indicates its own reliability, in that, if the test functions properly, the section or sections containing the controls should exhibit the expected positive or negative results.

Figure 13 shows a fifth preferred embodiment 86 of the test apparatus of the present invention, wherein a liquid sample or specimen 54 may be placed into the sample receiving area or well 32 through an appropriate, substantially horizontally disposed aperture 88 in the housing 22.

Figure 14 shows a sixth preferred embodiment 90. In this embodiment, one or two ducts or channels 44 fluidly interconnect with one another, before they reach the main sample receiving well 32. This embodiment is primarily useful in tests where two reagents 38 must be added substantially simultaneously, and should be well mixed with one another.

Figure 15 shows a seventh preferred embodiment 92. This embodiment 92 differs principally from the above-described embodiments (primarily the first preferred embodiment 20 which was described in detail) in that an empty chamber 94, capable of acting as a liquid reservoir, is located above the bottom plate 24 of the housing 22. The bottom plate 24 is flexible, and has an aperture 96 equipped with a one-way check valve 98 which allows air to exit from the chamber 94 when the flexible bottom plate 24 is compressed. The seventh

preferred embodiment 92 has no liquid absorbing padding or like material.

Thus, when it is desired to remove a liquid reagent from the sample receiving well 32 through the porous plate 56, an operator (not shown) manually compresses the bottom plate 24, whereby some air is expelled through the check valve 98 from the chamber 94 disposed below the porous plate 56. After the bottom plate 24 is released, the resulting vacuum in the chamber 94 "draws" the liquid reagent from the sample receiving well 32 through the porous plate 56 into the chamber 94. This process may be repeated several times with several liquid reagents, as they are applied one after another during the performance of the diagnostic or like test.

In light of the foregoing, in the seventh preferred embodiment of the device of the present invention, the chamber 94 in the housing 22 acts as a reservoir for used liquid reagents. The above-described feature of the seventh preferred embodiment also permits relatively fast removal of the liquid reagents from the sample receiving well 32.

Referring now to Figure 16, an eighth preferred embodiment 100 of the device of the present invention is disclosed. The eighth preferred embodiment 100 is a variation of the first preferred embodiment 20, and principally differs from the first preferred embodiment only in that a solid reagent 102 is affixed in the sample receiving well 32. The solid reagent 102 is, of course, one of the reagents which is required for the performance of the specific diagnostic or like test for which the specific test apparatus is designed. Those skilled in the art will readily recognize that the solid reagent 102 may be an enzyme, or an antibody, and that, in many instances, enzymes and other protein comprising reagents are more stable in a solid (lyophilized or the like) state than in solution. The solid reagent 102 may be affixed to the sample receiving well 32 as a tablet, as is shown on Figure 16, or may be otherwise deposited in the sample receiving well 32, as, for example,

in the form of a concentrated solution which is thereafter permitted to dry. As it will be readily recognized by those skilled in the art, in the tablet shown on Figure 16, the "active" solid reagent 102, such as an enzyme or antibody, is likely to be admixed with other inert or auxiliary solid materials, such as stabilizing agents or desiccants.

Referring now to Figures 17 and 18, still further embodiments in the test apparatus of the present invention are shown. In these embodiments, a solid reagent 102 is contained within a plastic bubble 36 which is disposed above one of the wells or depressions 34 formed in the intermediate member 28 of the housing 22. In the embodiment shown on Figure 17, the channel or duct 44 leads from yet another well 34 to the well containing the solid reagent 102. Therefore, when the liquid contents 38 of the plastic bubble 36 are released, the solid reagent 102 becomes exposed to the liquid reagent to be dissolved therein. The freshly created solution is then added to the main sample receiving well 32.

The embodiment shown on Figure 18 is similar to the embodiment shown on Figure 17, with the only substantial difference that liquid reagents are added from two plastic bubbles to the solid reagent 102 encased in a plastic bubble 36. It is noted in this regard that, in accordance with the varying requirements of specific tests in the embodiments shown on Figures 17 and 18, the ducts 44 and the respective plastic bubbles 36 may be formed in such a manner that the liquid reagent enters the plastic bubble which encases the solid reagent 102.

Although the specific chemical, clinical diagnostic, immunoassay, or like tests, to which the apparatus of the present invention may be adapted, constitute merely the application of the present invention, and not a part thereof, the following examples of specific tests, which are per se known in the art, are briefly described to further illuminate and explain the invention.

Thus, the known immunoassay test for human chorionic gonadotropin hormone (HCG) is described in the following

manner with reference to the first specific embodiment 20 of the test apparatus of the present invention. As is known, HCG is secreted in every increasing amounts by the human placenta after fertilization, so that the test is an indication of pregnancy.

In the test apparatus of the present invention adapted for the HCG test, the filter paper membrane 60 contains, bound to its cellulose fibers, a monoclonal antibody which reacts with and binds to the HCG molecule. The indicia or directions 30 describe the following steps, and the plastic bubble containers 36, sequentially numbered 1 through 4, contain the appropriate, below-described reagents or wash solutions 38.

In the first step of the procedure, a small, predetermined quantity of urine sample (of the human female subject) is placed on the filter paper 60 in the main sample receiving well 32. The urine sample is allowed to completely drain through the filter paper 60 and porous plate 56 into the underlying absorbant material 58. In this step, the HCG content of the urine (if there is any) binds to the monoclonal antibody, and is retained in the filter paper or membrane 60.

In the next step, the plastic bubble 36, identified with the arabic numeral 1, is depressed against the underlying spike 46 so that it ruptures, and its contents flow into the sample receiving well 32. This reagent, in the herein-described test for HCG, contains an antibody chemically linked to an enzyme. The reagent containing the antibody-linked enzyme is allowed to completely drain through the filter paper 60 and porous plate 56 into the absorbant material 58. The antibody binds to the HCG already bound on the filter paper or membrane 60. The enzyme, coupled to the antibody, is capable of causing a color reaction in a subsequent step of the assay. It follows from the foregoing that, if there is no HCG in the urine sample, there is no binding of the antibody-linked enzyme to the filter paper during this step.

In the next step, the plastic bubble 36, identified with the arabic numeral 2, is ruptured through pressing against the underlying spike 46. The reagent contained in this bubble 36 is a wash solution, such as saline. The wash solution is allowed to drain completely through the filter 60 and porous plate 56. The wash solution washes away any HCG which was not immobilized in the filter 60 by binding to the antibody contained therein. It also washes away any antibody-linked enzyme which was not immobilized by binding to the HCG molecules bound to the antibody immobilized on the filter 60.

In the subsequent step, the plastic bubble 36, identified as number 3, is ruptured to release a "color developer" reagent into the sample receiving well 32. The "color developer" reagent is a mixture of chemicals which react and develop, or change, color under the catalytic activity of the enzyme bound in the filter 60.

After a two-minute waiting period, the plastic bubble 36, identified as number 4, is ruptured to release a wash solution, and to wash away the "color developer" from the filter 60, thereby stopping the color development process. The intensity of color developed in the filter 60 is proportional to the amount of HCG hormone present in the urine sample. Lack of color indicates a negative, "not pregnant" result.

Readily apparent advantages of the test apparatus of the present invention are the simplicity and ease of procedure for performing the above-described, and like, otherwise relatively cumbersome and attention demanding, diagnostic and similar tests.

Another advantage is that the reagents are separated from one another, and not mixed until immediately before the test. In some instances, this is particularly important, as, for example, in the well-known "Trinder methodology" for cholesterol determination. In this methodology, free cholesterol is oxidized in the presence of cholesterol oxidase enzyme to cholesten-3-one with the production of

hydrogen peroxide. Hydrogen peroxide reacts, in the presence of peroxidase enzyme, with 4-amino anti-pyrine and phenol to yield a spectrophotometrically measurable colored dye. However, the mixture of 4-amino anti-pyrine and phenol is not stable in aqueous solutions. Therefore, in the prior art, the reagent kits for the Trinder methodology contained lyophilized mixtures of these two reagents, which had to be reconstituted shortly before use.

In a test apparatus adapted in accordance with the present invention for the Trinder methodology, the aqueous solution of phenol is kept in a separate container from the aqueous 4-amino anti-pyrine, whereby the problem of low stability is eliminated, and there is no need for relatively expensive lyophilized reagents.

It will be readily apparent, to those skilled in the art, that several modifications in the construction and application of the test apparatus of the present invention can be made in light of the foregoing disclosure. Therefore, the scope of the present invention should be interpreted solely from the following claims, as such claims are read in light of the disclosure.

WHAT IS CLAIMED IS:

1. An apparatus adapted for receiving a sample or specimen on which a chemical or diagnostic test is to be performed, for containing a predetermined amount of at least one liquid reagent which is to be used for performing the test, and for facilitating the addition of the reagent to the sample, the apparatus comprising:

a housing;

a liquid reservoir area defined in the housing and comprising means for receiving the sample or specimen;

at least one container at least partially contained in the housing, the container containing a predetermined amount of the liquid reagent required for performing the test, and having rupturable walls;

means contained in the housing for rupturing the wall of the container, to cause the liquid reagent to escape from the container, and

means connected with the liquid reservoir area for permitting the liquid reagent which has escaped from the container to flow to the liquid reservoir area and to the sample or specimen thereon, whereby the liquid reagent contents of the container is added to the sample or specimen.

2. The apparatus of Claim 1 wherein the container comprises a sealed plastic bubble, and wherein the means for rupturing comprise means included in the housing for piercing the plastic bubble when the bubble is pressed against the means for piercing.

3. The apparatus of Claim 3 wherein the means for rupturing comprise a sharp point included in the housing and disposed substantially below the plastic bubble.

4. The apparatus of Claim 1 wherein the container comprises a sealed plastic bubble, and wherein the means for rupturing comprise a weakened wall section incorporated in the plastic bubble, the weakened wall section being adapted for rupturing under pressure when sufficient pressure is manually applied by an operator against the plastic bubble.

5. The apparatus of Claim 1 further comprising a sheet of plastic material contained in the housing, and wherein the container comprises a sealed plastic bubble and the bubble is attached to the sheet.

6. The apparatus of Claim 1 wherein the liquid reservoir area is defined by a first depression incorporated in the housing, wherein a further depression is defined by the housing disposed below the container, and wherein the means for permitting comprise a duct incorporated in the housing, said duct establishing fluid communication between the first depression and the depressions disposed below the container.

7. The apparatus of Claim 6 further comprising a sheet of plastic material contained in the housing, and wherein the container comprises a sealed plastic bubble and the bubble is attached to the sheet, and further wherein said duct is formed as an upwardly open channel in the housing, and the sheet comprises an upper closure member for the duct.

8. The apparatus of Claim 1 further comprising a sheet of plastic material contained in the housing, and wherein the container comprises a sealed plastic bubble and the bubble is attached to the sheet in a predetermined geometrical configuration, and further wherein the housing comprises an upper plate and a lower plate, the upper plate having an opening, said opening providing manual access to the plastic bubble, whereby manual pressure may be applied by an operator on the plastic bubble for rupturing the plastic bubble through the means for rupturing.

9. The apparatus of Claim 9 wherein the means for rupturing comprise means included in the housing for piercing the plastic bubble when the bubble is pressed against the means for piercing.

10. An apparatus to be used in connection with the performance of a medical diagnostic test and the like, wherein a sample or specimen is exposed to a plurality of liquid reagents in a predetermined sequence, the apparatus comprising:

a housing having a bottom plate and a top plate, said plates being assembled to one another and defining an, at least partially, enclosed interior space, the upper plate having a plurality of openings;

a liquid reservoir area defined within the interior space, said liquid reservoir area being included in a depression formed within the housing and being disposed below one of the openings in the upper plate, the depression and the liquid reservoir area being adapted to contain the sample or specimen;

a plurality of recesses defined within the housing, each of said recesses being disposed in the interior space at least partially below one opening in the upper plate;

a plurality of sealed containers having rupturable walls, each of the containers being located above one of the recesses and containing a predetermined amount of liquid reagent which is used in the diagnostic test;

duct means incorporated in the housing for fluidly connecting each of the recesses with the first liquid reservoir area, and

means for selectively rupturing any one of the sealed containers at the option of an operator, whereby the liquid reagent is released from the ruptured container into the corresponding recess and is transferred therefrom to the liquid reservoir area through the duct means so as to expose the sample or specimen to the liquid reagent, and whereby the sample or specimen is exposed to the liquid reagents in the sequence in which the operator causes rupturing of the sealed containers.

11. The apparatus of Claim 10 wherein the sealed containers comprise plastic bubbles.

12. The apparatus of Claim 11 wherein the means for selectively rupturing comprise members integral with the housing disposed in the recesses and having a sharp edge

comprising means for piercing the plastic bubble when the plastic bubble is pressed against the sharp edge.

13. The apparatus of Claim 11 wherein each plastic bubble has a narrow projection, and wherein the means for selectively rupturing comprise means, manually actuable by the operator, for shearing off the narrow projection.

14. The apparatus of Claim 11 wherein the means for selectively rupturing comprise a weakened wall section incorporated in each of the plastic bubbles, the weakened wall section being adapted for rupturing when sufficient manual pressure is exerted on the plastic bubble.

15. The apparatus of Claim 11 further comprising a plastic sheet, the plastic bubbles being fixedly mounted to the plastic sheet in a predetermined configuration.

16. The apparatus of Claim 11 further comprising liquid absorbing means disposed below the liquid reservoir area for at least partially absorbing the liquid reagents used in the test.

17. The apparatus of Claim 16 further comprising a porous plate disposed below the liquid reservoir area and above the liquid absorbing means.

18. The apparatus of Claim 17 further comprising filter paper disposed in the liquid reservoir area above the porous plate.

19. The apparatus of Claim 11 further comprising means for removing by suction liquid reagent from the liquid reservoir area into a waste liquid reservoir space defined in the housing below the liquid reservoir area.

20. The apparatus of Claim 19 wherein the means for removing by suction comprise a flexible, compressible plate incorporated in the housing and valve means for releasing air into the ambient from the waste liquid reservoir space when the flexible plate is compressed.

21. An apparatus to be used in connection with the performance of a medical diagnostic test and the like, wherein a sample or specimen is exposed to a plurality of

liquid reagents in a predetermined sequence, the apparatus comprising:

a housing having a bottom plate and a top plate, said plates being assembled to one another and defining an at least partially enclosed interior space, the upper plate having a plurality of openings;

a first depression formed in an interior surface of the housing below a first opening, the first depression forming a liquid reservoir and space for placement of a sample or specimen on which the diagnostic or like test is to be performed;

a plurality of additional depressions formed in the interior surface of the housing, each additional depression being disposed below one of the openings in the upper plate;

a plurality of ducts connecting each additional depression with the liquid reservoir and establishing fluid communication from the respective additional depression to the liquid reservoir;

a plurality of plastic bubbles, each bubble comprising a sealed container for a predetermined amount of liquid reagent which is used in the diagnostic or like test, each bubble being contained at least partially in the housing above one additional depression, and

a plurality of members disposed at least partially in the housing, each member being in operative association with one of the plastic bubbles and comprising means for rupturing the respective plastic bubble, whereby the liquid reagent is released from the plastic bubble and flows into the liquid reservoir to come into contact with the sample or specimen.

22. The apparatus of Claim 21 wherein each member is integrally constructed with the housing and has a sharp projection disposed toward a plastic bubble, each member being located in one of the additional depressions, whereby pressure applied manually against any of the plastic bubbles

through the respective opening forces the plastic bubble against the sharp projection and pierces the plastic bubble.

23. The apparatus of Claim 22 further comprising liquid absorbing means disposed below the liquid reservoir area for at least partially absorbing the liquid reagents used in the test.

24. The apparatus of Claim 23 further comprising a porous plate disposed below the liquid reservoir area and above the liquid absorbing means.

25. The apparatus of Claim 24 further comprising a filter member disposed in the liquid reservoir area above the porous plate.

26. The apparatus of Claim 25 wherein the filter means contains a bound antibody necessary for the test.

27. The apparatus of Claim 21 further comprising an enclosed waste liquid reservoir space disposed below the liquid reservoir area and means for drawing by suction liquid reagent from the liquid reservoir area into the liquid reservoir space.

28. The apparatus of Claim 27 further comprising a porous plate disposed below the liquid reservoir area and above the waste liquid reservoir space.

29. The apparatus of Claim 28 wherein the means for drawing comprises a flexible compressible plate of the housing and valve means for releasing air from the waste liquid reservoir space when the flexible plate is compressed.

30. The apparatus of Claim 21 comprising a peelable band attached to the top plate of the housing, the peelable band comprising means for preventing access to the plastic bubbles until the peelable band is at least partially removed from the upper plate.

31. The apparatus of Claim 30 wherein the peelable band carries indicia containing instructions for performing the test.

32. A process for performing a diagnostic or like test on a sample or specimen, said test requiring the addition of substantially predetermined volumes of a plurality of liquid

reagents to the sample or specimen in a predetermined sequence, the process comprising the steps of:

placing the sample or specimen into a liquid reservoir maintained in a housing;

maintaining a predetermined volume of each one of the liquid reagents in a sealed plastic bubble arranged in a predetermined configuration in the housing, said housing or said bubbles having means for rupturing each of the plastic bubbles;

rupturing each of the plastic bubbles in the predetermined sequence required by the diagnostic or like test, and

immediately after each step of rupturing, permitting the predetermined volume of liquid reagent to flow into the liquid reservoir to come into contact with the sample or specimen.

33. The process of Claim 32 wherein each step of rupturing comprises piercing the respective plastic bubble with a sharp object.

34. The process of Claim 33 wherein each step of piercing includes a step of pressing the plastic bubble downwardly against the sharp object which is contained in the housing below the respective plastic bubble.

35. The process of Claim 32 wherein each step of rupturing includes a step of shearing a narrow neck portion of the respective plastic bubble.

36. The process of Claim 32 wherein each step of rupturing includes a step of applying sufficient pressure on the respective plastic bubble to cause internal pressure of the liquid reagent contained in the bubble to rupture a weakened wall section of the bubble.

37. The process of Claim 32 including the additional step of waiting for a predetermined period of time after rupturing at least one of the plastic bubbles.

38. The process of Claim 32 including the additional step of removing by suction the liquid reagent from the

liquid reservoir after the liquid reagent has come into contact with the sampl or specimen.

39. The process of Claim 38 wherein the step of removing by suction comprises removing the liquid reagent into the interior of the housing.

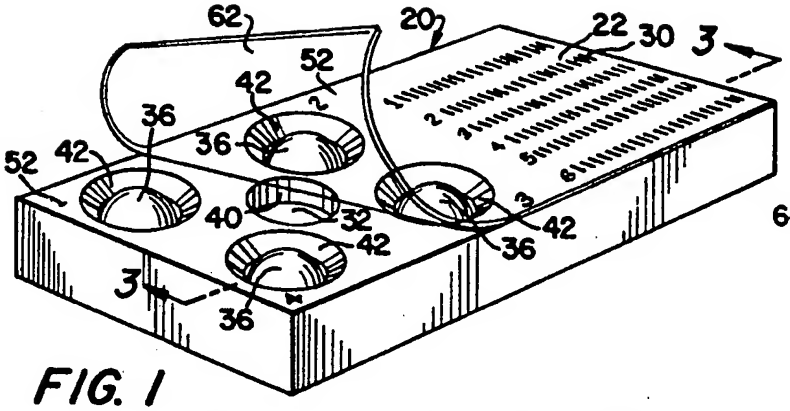


FIG. 1

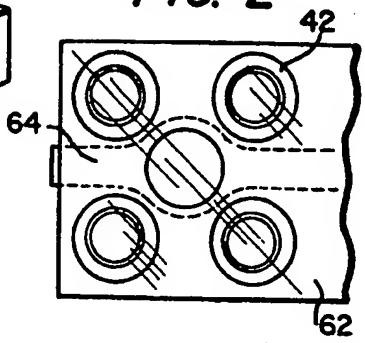


FIG. 2

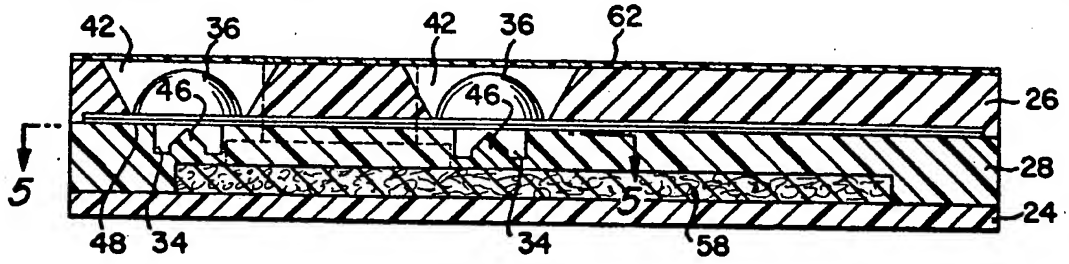


FIG. 3

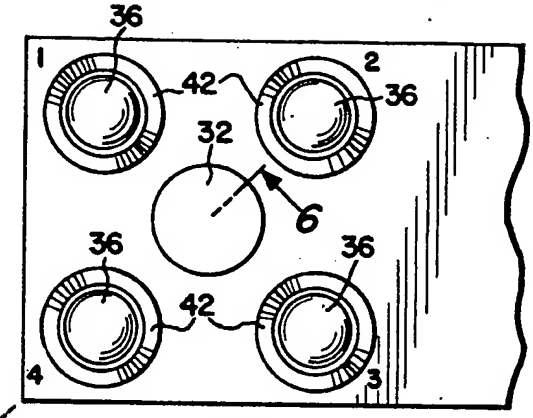


FIG. 4

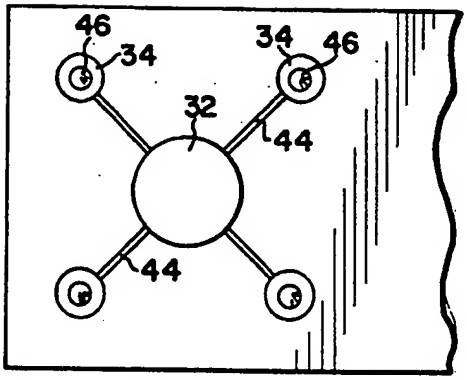


FIG. 5

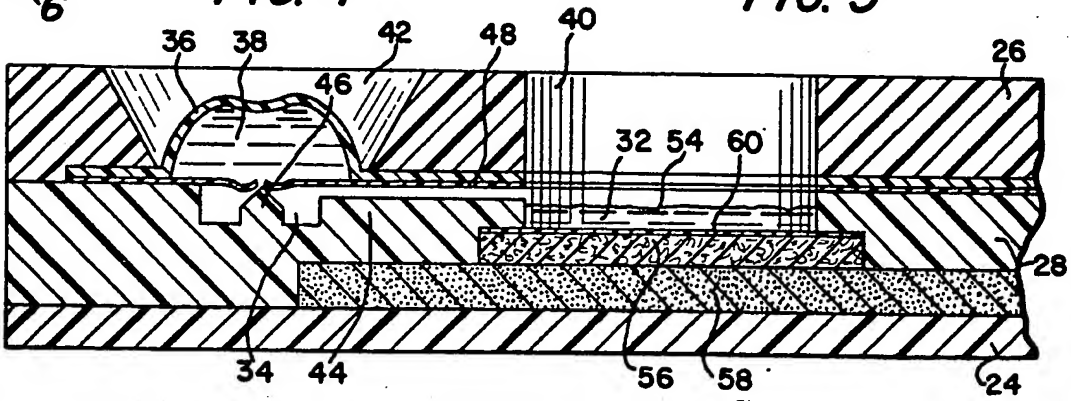


FIG. 6

FIG. 7

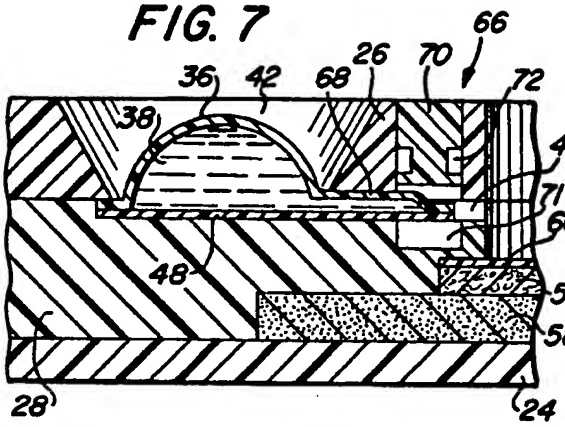


FIG. 8

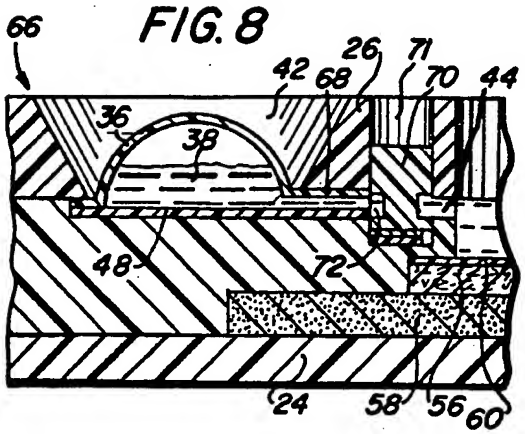


FIG. 9

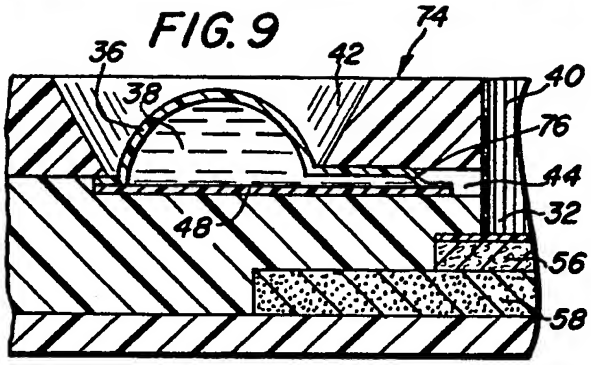


FIG. 10

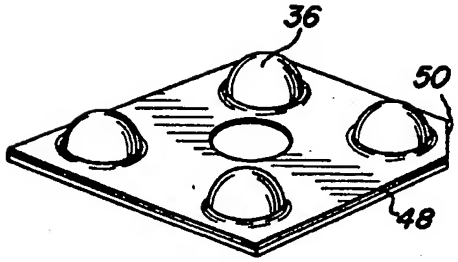


FIG. 11

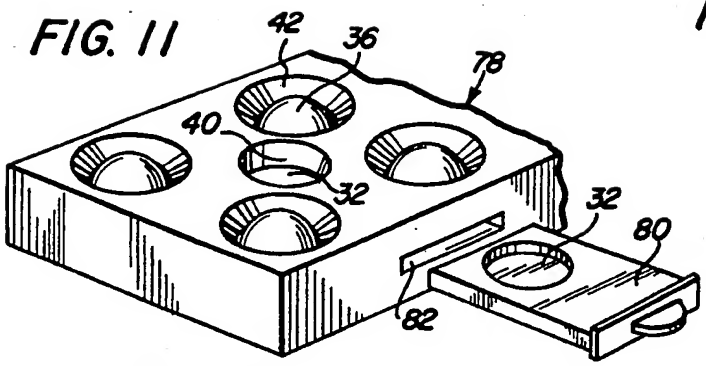
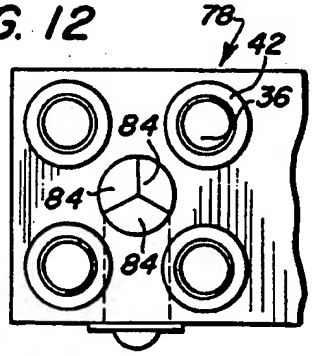


FIG. 12



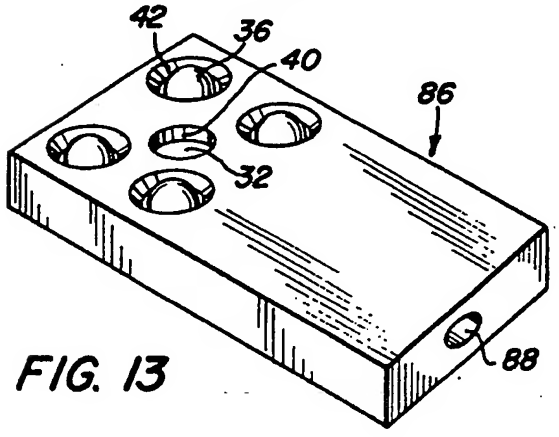
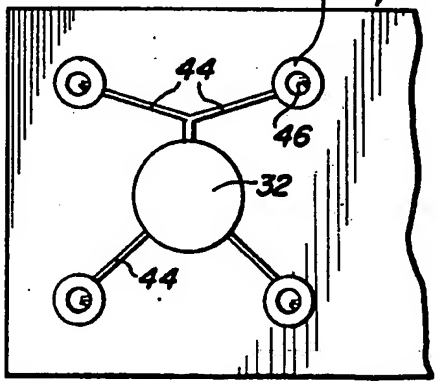


FIG. 13

FIG. 14



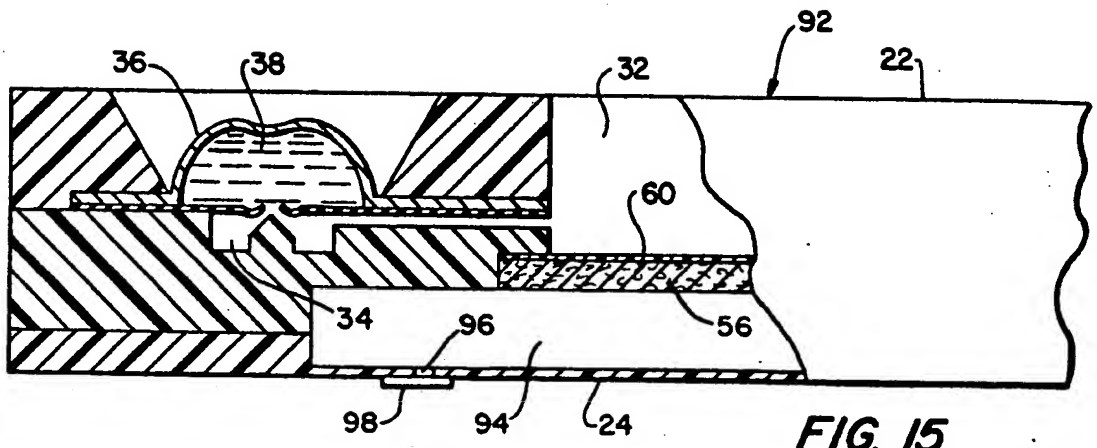


FIG. 15

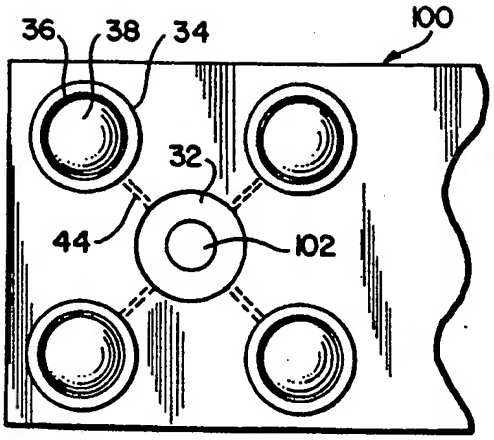


FIG. 16

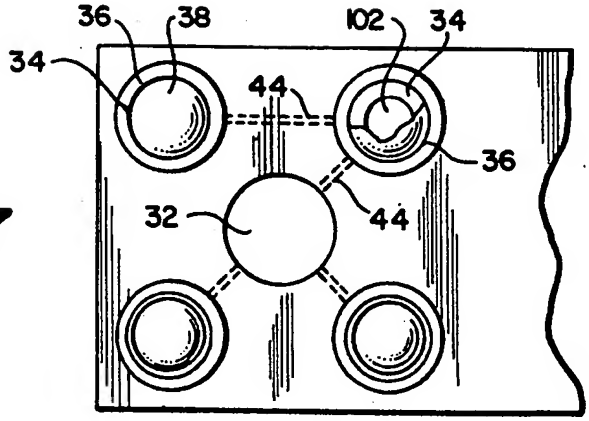


FIG. 17

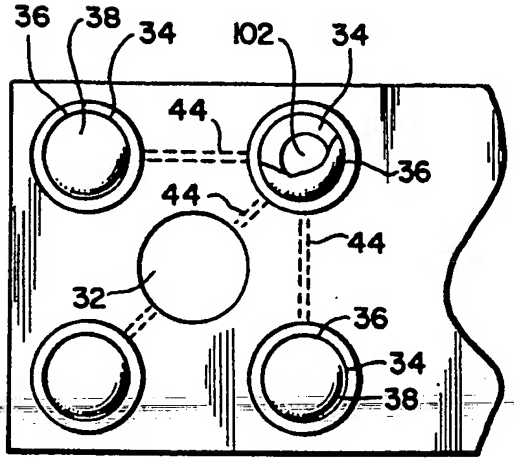


FIG. 18

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/00709

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. 435/4, 7.288,292.293.294,300,301.810; (See Attachment)
INT. Cl. [4] G 01 N 33/53, 33/543, 33/544; C 12 Q 1/00;

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/4,7,288,292,293,294,299,300,301,810 436/518,528,530,535,808,809

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

HICHEM DIAGNOSTICS, INC., DBA BURAL TECHNOLOGIES.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US,A, 4,324,758 (EISENTRAUT ET AL) 13 April 1982 See column 8. line 26 to column 9, line 13.	1.4,6,10, 11,14,32, 36,37 1-39
X Y	US,A, 3,497,320 (BLACKBURN ET AL) 24 February 1970 See column 5, line 69 to column 7, line 11.	1.4-7, 32,36 1-39
X	US,A, 3,689,224 (AGNEW ET AL) 05 September 1972 See column 4. lines 63 to 68.	1.4,5.6
X	US,A, 3,476,515 (JOHNSON ET AL) 04 November 1969 See column 1. lines 15 to 26.	1,4
Y	US,A, 3,888,629 (BAGSHAWE) 10 June 1975 See column 1, line 26 to 43.	16-19,23-28.38.39

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
09 JUNE 1986	26 JUN 1986
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Randall E. Deck

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INF RMATION CONTINUED FR M THE SECOND SHEET

Y	US,A,	4,246,339 (COLE ET AL) 20 January 1981 See column 1, line 52 to column 2, line 35.	16-19,23-28,38,39
Y	US,A,	4,428,907 (HEIJENGA ET AL) 31 January 1984 See column 4, line 67 to column 5, line 5.	2,3,9,12,13,21,22,33-35

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No 18
Y	US,A, 3,799.742 (COLEMAN) 26 MARCH 1974, See column 13, lines 32 to 39.	2,3,9.12, 13,21,22, 33-35
Y	US,A, 3,768,974 (STORM) 30 October 1973 See column 1, line 60 to column 2, line 11	19,20,27-29,38.39
Y	US,A, 3,740,196 (STOTERHOFF) 19 June 1973 See column 3, lines 9 to 11.	30,31
A	US,A, 4,090,850 (CHEN ET AL) 23 May 1978.	19,27,28, 38,39
A	US.A, 4,407,943 (COLE ET AL) 04 October 1983.	19,27,28, 38,39
A	US,A, 4,458,020 (BOHN ET AL) 03 July 1984.	1-39
A	WO,A1, 8202211 (CHANDLER ET AL) 08 July 1982.	1-39
A	DE,A, 2,028,822 (KELLER) 16 December 1971.	1-39

PCT/US86/00709

I. Classification of Subject Matter (continued)

U.S. 436/518, 528, 530, 535, 808, 809
422/56, 58, 60, 61, 99, 102
206/569, 305
435/299
INT. Cl. 4
C12M 1/16, 1/18, 1/20, 1/26, 1/32, 1/40, 1/28
B01L 3/00

II. Fields Searched

422/56, 58, 60, 61, 99, 102; 206/569, 305